QUALIFIED HEALTH CLAIM PETITION

100% WHEY PROTEIN PARTIALLY HYDROLYZED in Infant Formula and REDUCING THE RISK OF ALLERGY IN INFANTS

ANALYTICAL DATA – SUBSTANCE CHARACTERIZATION

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INTRODUCTION

Section 101.70(f) provides that a health claim petition should contain analytical data that show the amount of the substance that is present "in representative foods that would be candidates to bear the claim" and that such data "should be obtained from the representative samples using methods from the Association of Official Analytical Chemists (AOAC), where available." The section goes on to provide that if no AOAC method is available, the petition must contain the assay method used and data establishing the validity of the method for assaying the substance in food.

Although critical to both establishing and policing many health claims, the analytical data requirement in the context of this petition is somewhat anomalous in light of the fact that only one food, infant formula, is at issue and the amount of protein in that food is restricted within a statutorily prescribed range (1.8 – 4.5 g/100 kcal). This range is further narrowed by current recommendations from the expert medical community; 1998 recommendations from an expert panel under the auspices of the Life Sciences Research Organization were for no more than 3.4 g/100kcal.

More relevant to the claim in question is the need to assure that any 100% Whey Protein Partially Hydrolyzed (PHF-W) used in infant formula, for which such a claim may be made, is consistent with the analytical attributes of the PHF-W used in the clinical studies that support this proposed claim. The following section discusses all those analytical attributes, as well as describing the analytical method relied upon by Nestlé to determine the precise amount of protein.

ANALYTICAL ATTRIBUTES OF THE SUBSTANCE

As discussed in Section C: Summary of Scientific Data, a large body of evidence supports the proposed claim regarding the link between the exclusive feeding of 100% Whey Protein Partially Hydrolyzed in infant formula and a reduction in the risk of allergy. Should FDA authorize a health claim regarding this link, it is important that it be applied only to formulas containing the studied substance. The main purpose of this section of the petition is to characterize this substance in such a way that FDA, and manufacturers who wish to make use of such a claim, may determine whether a given product contains the eligible substance.

It should be noted, first of all, that all hydrolyzed formulas are not the same. For example, a considerable amount of data support the use of extensively hydrolyzed cow's milk protein in formulas (EHF) to reduce the risk of allergy, in addition to the therapeutic use for which they were designed. And, although the body of evidence on EHF for use in primary prevention is smaller than that collected on PHF-W, EHF are already considered useful by the medical community in reducing the risk of allergy in those infants identified as being at "high risk", based on their family history of atopic disease. Unfortunately, the much greater expense of these formulas, in addition to their lack of palatability, limits their use in the general population. Since FDA-authorized health claims are intended for the general population, this petition is not intended to cover extensively hydrolyzed cow's milk protein in infant formulas.

To a limited degree, partially hydrolyzed casein/whey protein blends in infant formula (PHF-C/W) have also been studied for use in primary prevention (Szajewska

2004, Han 2003, Oldaeus 1997). However, only one study to date has shown a statistically significant effect on allergy prevention (Han 2003), and the interpretation of these results is limited by the study's short-term follow-up (6 months). Consequently, this petition is not intended to cover partially hydrolyzed casein/whey protein blends in infant formulas.

Of the total of 13 study cohorts in which data on PHF-W in allergy prevention have been collected, only one (Chirico 1997) used a PHF-W from another manufacturer. All other studies of PHF-W used what Nestlé refers to as its "HA" formulations. In spite of slight variations over the years in levels and sources of protein, processing conditions, etc., these formulations have consistently been shown to reduce the risk of allergy. Thus, Nestlé proposes that the characteristics of the substance eligible to carry the proposed claim be defined in terms of the functional substance common to all of the Nestlé HA formulations tested – i.e., a protein base for infant formula that is characterized by meeting all of the technical criteria detailed in the following pages. These criteria are discussed in order of their ease of use in eliminating products that are not sufficiently similar to the studied formulas to justify their carrying the proposed claim.

Protein

The first criterion for identifying an eligible infant formula product should be a determination of the type of protein used. The protein component in an eligible formula would consist of 100% Whey Protein in accordance with the description of "Whey protein concentrate" at 21CFR 184.1979c (attached at Appendix D-I), which may or may not be further isolated (e.g., to reduce mineral or lactose content) and which may or may not be fortified with additional amino acids. The analytical method relied upon by Nestlé to

determine the amount of protein (via total nitrogen) is equivalent to AOAC method #991.20. However, as noted above, the *amount* of protein in infant formula is already sufficiently constrained by statute and by current medical recommendations. What is important from the point of view of determining eligibility for the proposed claim is the *type* of protein, and that only protein in compliance with the 21CFR 184.1979c definition be used in the manufacture of the formula. If this criterion were not met in a given product, there would be no basis for associating that product with the clinical substantiation for this claim, and so no additional criteria would need to be considered.

Enzyme

The second criterion for determining eligibility should be an examination of the method of hydrolysis, beginning with the type of enzyme used. The protein component in the studied formulas is hydrolyzed with Porcine Trypsin, as described in 21 CFR 184.1914 (attached at Appendix D-II), an enzyme preparation obtained from purified extracts of porcine pancreas. The enzyme must have both trypsin and chymotrypsin activity, and not other proteases which could potentially yield peptides significantly different than those resulting from physiologic pancreatic digestion.

Process

Any further examination of potential eligibility should focus on the hydrolysis process. The protein in the studied formulas was hydrolyzed by a process that includes hydrolysis of the whey protein described above with the enzyme described above to provide a first enzymatic hydrolysate; heating the first hydrolysate for 3 to 10 minutes at a temperature of 80 degrees C to 100 degrees C at pH 6 to 8 to denature proteins remaining

intact after the first hydrolysis; cooling the heated first hydrolysate to a temperature of 40 degrees C to 60 degrees C; subjecting the cooled first hydrolysate to hydrolysis with the enzyme described above to hydrolyze the intact denatured proteins in the first hydrolysate for providing a second hydrolysate substantially free of allergens of protein origin; and then heating the second hydrolysate to thermally inactivate the enzyme for providing a substantially allergen-free hydrolysate product.[1]

Finished Product Characteristics

Only if a product met all of the first three criteria for eligibility, would it be necessary to go on to confirm their similarity to the studied formulas through analysis of the end product of the formula's manufacture. The typical end product resulting from this process can be described as falling within the following ranges on the following indicators of appropriate hydrolysis:

NPN/TN % - Non-Protein Nitrogen over Total Nitrogen is widely used as a measure of soluble protein created by enzyme hydrolysis. In the 100% Whey Protein Partially Hydrolyzed infant formulas (PHF-W) used in the clinical trials discussed in this petition, NPN/TN typically ranges between 71 – 93%. The analytical method used to measure NPN is equivalent to AOAC method #991.21. TN is determined by the method referred to above for the measurement of total protein.

Alpha amino-N/TN % – Alpha amino-Nitrogen over Total Nitrogen is another widely used as a measure of degree of hydrolysis. As late as 1990, Nestlé shared with FDA its observations that Good Start (now called Good Start Supreme) was 17.5%

[1] This process description is adapted from Claim 1 of United States Patent Number 5,039,532 dated August 13, 1991.

hydrolyzed. Similarly, a European patent owned by Nestlé (filed in 1996) describes the HA formulations as 15-19% hydrolyzed. The values seen currently in these formulas are typically between 9 – 14 %. The difference between the earlier values and the current ones is due to the fact that the earlier values were measured by the "ninhydrin" method. This same degree of hydrolysis, as currently measured by Nestlé worldwide using the newer TNBS method, results in a lower numerical range. The TNBS method for measuring Alpha amino-Nitrogen over Total Nitrogen may be found under Appendix D-III.[2] It should be noted here that, according to the European patent, in order to induce "oral tolerance" – which is theorized to be the mechanism by which the HA formulations reduce the risk of allergy – it is as important for the degree of hydrolysis not to be too high, as it is for it not to be too low.

BLG – Nestlé tests Beta-lactoglobulin (BLG) periodically, using the ELISA method, as an indicator of total immunoreactive material. BLG constitutes somewhere between 30% - 50% of the total immunoreactive material in whey. Thus a BLG level below 3 mg/g protein, which is typical of the HA formulations, indicates that the product has no more than 1% immunoreactive protein. Such a level is consistent with Annex IV of European Commission Directive 91/321/EEC of 14 May 1991 on Infant Formulae and Follow-On Formulae (attached at Appendix D-IV, see p.19, item 7), which sets forth certain conditions for an infant formula claiming to reduce the risk of allergy. The first condition is that the maximum for immunoreactive protein would be 1% of nitrogen

[2] The method for TN in this Nestlé laboratory instruction is referenced as LI-00.556, which is equivalent to AOAC 991.20

containing substances in the formula.[3] That is equivalent to a total of 10 mg of immunoreactive protein (as BLG) per g nitrogen containing substance (as protein). The analytical method Nestlé uses to measure BLG may be found under Appendix D-V.

Molecular Weight & Peptide Profile – The median molecular weight of 100% Whey Protein Partially Hydrolyzed infant formulas (PHF-W) is typically between 1000 – 1350 Daltons. Attached at Appendix D-VI is a graphic illustration of how PHF-W compare on this parameter with a standard intact cow's milk formula. Also under Appendix D-VI is a typical mean molecular weight distribution for these PHF-W. 50% or more of the peptides have a mean molecular weight of less than 1,500 daltons, and less than 5% of the peptides greater than 5,000 daltons. Nestlé's method for peptide profiling by size exclusion chromatography is enclosed at Appendix D-VII.

Release Criteria – While the above criteria are useful in determining the general similarity to the studied formulas, only two specific release criteria have been deemed reliable enough to remain in effect, both globally and from the time these formulas were introduced, among the Nestlé PHF-W demonstrated as effective in the clinical trials described in Section C: Summary of Scientific Data. These criteria require performance consistent with a control on two tests of hydrolysis: SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Immunodiffusion. These are semi-quantitative visual comparisons, not subject to numerical description. These two analytical methods may be found under Appendices D-VIII and D-IX.

[3] The second criterion requires that the product label indicate that it is not intended for use in infants allergic to cow's milk unless the product has been clinically proven to be tolerated by 90% of such infants. This level of tolerance is roughly equivalent to the AAP criterion for use of the term "hypoallergenic". Accordingly, any product meeting the substance definition for this claim must be labeled as not for use in infants allergic to cow's milk. The final two criteria are discussed below.

Preclinical Indicators of Functionality

Even where a potentially eligible formula has met all the preceding criteria, the agency may wish to further ensure its protective effect against allergy through the use of some indicator of clinical functionality. Nestlé has found the following preclinical testing methods useful in this regard.

As mentioned in the section above on BLG, the protein hydrolysis process used in manufacturing PHF-W significantly reduces the immunoreactive protein content (>99% compared to intact protein) without eliminating it completely. And, in addition to the BLG testing described above, the residual BLG-specific allergenicity has also been determined *functionally* by tritiated serotonin (3H serotonin) release assay.

The level of BLG-specific IgE mediated allergenicity of PHF-W has been determined by evaluating the capacity of this formula to trigger the release of serotonin (an allergic mediator) from sensitized mast cells. To test this, peritoneal mast cells from normal rats were labeled with 3H serotonin and sensitized passively in vitro with a pool of rat sera containing specific IgE anti-BLG. Then, 3H serotonin release was triggered with increasing concentrations of bovine BLG (as a standard) or with PHF-W or intact cows' milk protein infant formula (CMF). The dose-dependent response in 3H serotonin release (shown in Fig. I at Appendix D-X) has been previously documented. Significantly more PHF-W is required, on a protein equivalent basis, to trigger similar amounts of 3H serotonin compared to CMF (Fritsché 1997). The residual BLG-specific allergenicity calculated based on this functional assay is 0.39% to 0.5% of that found in CMF. These

values are consistent with the corresponding values of BLG as determined by the ELISA method.

Using a similar animal model, consumption of PHF-W have also been demonstrated to induce suppression of IgE antibody as well as suppression of mast cell serotonin release, as indicators of allergic response. In other words, intake of PHF-W leads to immunomodulatory mechanisms, which can decrease the allergic response to cow milk proteins (such as BLG) by inducing immunologic "tolerance" to these proteins.

In these experiments, rats were orally fed either PHF-W or water (as placebo) as part of their diet for a period of time. Then they were sensitized, by subcutaneous administration, with BLG, or with ovalbumin (a different unrelated protein, to assess the specificity of any immunomodulation associated to the oral intake of PHF-W). Two weeks later, specific IgE and IgG to BLG and ovalbumin were determined by ELISA. Fig. II (also at Appendix D-X) shows that preventive oral administration of PHF-W suppresses production of anti-BLG IgE and IgG in animals that were sensitized to BLG by injection. Specific serum IgE and IgG antibody production was suppressed by a factor of at least 25 (2 log5 ELISA antibody titers) compared to IgE secretion in the control rats that were fed placebo (water) (Fritsché 1990). In contrast, antibody responses to ovalbumin remained unchanged when compared to the control group given water, indicating that the suppressive, or "tolerizing" mechanism is antigen specific.

Using the same animal model, suppression of *in vitro* mast cell serotonin secretion has also been studied as another way to evaluate the tolerizing capacity of PHF-W (Fritsché 1997). Normal peritoneal mast cells were labeled with 3H serotonin and

passively sensitized with the sera of rats that had orally consumed PHF-W or water. Then, 3H serotonin release was triggered using increasing amounts of BLG. As expected, the mast cells from rats given water show a dose-dependent curve of serotonin release when exposed to increasing amounts of BLG, consistent with an allergic response. On the other hand, the serotonin release from mast cells sensitized with sera from rats fed PHF-W was completely blunted (see Fig. III, Appendix D-X).

In summary, PHF-W not only has reduced antigenicity, as demonstrated by a reduction in immunoreactive protein content; but in *in vivo* animal experiments, PHF-W leads to functional suppression of allergic response as measured by lower IgE antibody production and suppression of release of allergic reaction mediator (serotonin) from mast cells. These studies suggest that the consumption of PHF-W not only decreases the chances of allergic reaction by providing a lower antigenic load, but potentially also by promoting immunologic tolerance to cow's milk antigens.

This data satisfies the third criterion under the EC Directive described earlier for formulas that claim to reduce the risk of allergy: "the formulae administered orally should not induce sensitization, in animals, to the intact proteins from which the formulae are derived."

Clinical Evidence of Functionality

The fourth and final criterion under the EC Directive is that "objective and scientifically verified data as proof to the claimed properties must be available." Accordingly, any product making this claim must be shown, through meeting *all* of the technical characteristics described in this Section D: Analytical Data – Substance

Characterization, to be equivalent to the formulas used in the body of scientific evidence described in Section C: Summary of Scientific Data. The best evidence would, of course, be accumulated data from several clinical trials using the potentially eligible formula, as have been presented here for the Nestlé HA formulations.

APPENDICES